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Term: L22 and butanol

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Set Name side by side	Query	Hit Count	Set Name result set
DB=USPT,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ			
L24	L23 and betaine	0	L24
L23	L22 and butanol	6	L23
L22	(separat$ or extract$) near5 labeled near5 (DNA or nucleic acid) near5 (quencher or chelat$ or solid)	19	L22
L21	(fragmentation or cleav$) near5 metal near5 (label$ or attach$) near5 (nucleic acid or DNA)	9	L21
L20	L18 and thiophosphate$1	23	L20
L19	L18 and (DNA near5 thiophosphate)	0	L19
L18	l14 and (label$ or attach$ or link$)	133	L18
L17	l16 and (isolat$ or purif$ or extract$)	23	L17
L16	L15 and thiophosphate	23	L16
L15	L14 and label$	121	L15
L14	(fragment$ or cleav$) near5 (DNA or nucleic acid$1) near5 metal	148	L14
L13	L12 and metal and label$	2	L13
L12	l11 and (fragment$ or cleav$ near5 DNA)	2	L12
L11	6211356.pn.	2	L11
L10	label$ near5 DNA near5 (fragmentation or cleav$) near5 metal	1	L10
L9	label$ near5 DNA near5 (fragmentation or cleav$)	571	L9
L8	L7 and (unattaced or unlabeled)	5	L8
L7	l5 and (remov$ or decreas$ or eliminat$)	29	L7
L6	L5 and ((remove$ or decreas$ or eliminat$) near5 (unlabeled or unattached))	0	L6
L5	L4 and (thiol derivative or chelating agent or pyrophosphate)	29	L5
L4	L3 and label$	57	L4
L3	(fragmentat$ or cleav$) near5 DNA near5 metal	71	L3
L2	L1 and label$	0	L2
L1	fragmentation near5 DNA near5 metal	1	L1

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 6 of 6 returned.

☐ 1. 5624802 . 07 Jun 95; 29 Apr 97. Nucleic acid multimers and amplified nucleic acid hybridization assays using same. Urdea; Michael S., et al. 435/6; 435/7.1 435/810 436/501 536/22.1 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C07H021/04.

☐ 2. 5614362 . 30 Sep 94; 25 Mar 97. Nucleic acid hybridization assay for hepatitis B virus DNA. Urdea; Michael S., et al. 435/5; 435/243 435/320.1 435/6 435/948 436/501 436/811 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68.

☐ 3. 5594118 . 10 May 95; 14 Jan 97. Modified N-4 nucleotides for use in amplified nucleic acid hybridization assays. Urdea; Michael S., et al. 536/23.1; 435/5 435/6 435/91.1 536/24.3. C07H021/02 C07H021/04 C12Q001/68 C12Q001/70.

☐ 4. 5571670 . 14 Dec 93; 05 Nov 96. Nucleic acid probes useful in detecting Chlamydia trachomatis and amplified nucleic acid hybridization assays using same. Urdea; Michael S., et al. 435/6; 435/810 436/501 536/22.1 536/23.1 536/24.1 536/24.3 536/24.31 536/24.32 536/24.33. C12Q001/68 C07H021/04.

☐ 5. 5359100 . 13 Aug 93; 25 Oct 94. Bifunctional blocked phosphoramidites useful in making nucleic acid mutimers. Urdea; Michael S., et al. 552/105; 549/220 558/185. C07F009/24.

☑ 6. 5124246 . 18 Apr 89; 23 Jun 92. Nucleic acid multimers and amplified nucleic acid hybridization assays using same. Urdea; Michael S., et al. 435/6; 435/810 436/501 536/23.1 536/24.3 536/24.31 536/24.32. C12Q001/68 C07H021/00.

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Term	Documents
BUTANOL.DWPI,EPAB,JPAB,USPT.	74646
BUTANOLS.DWPI,EPAB,JPAB,USPT.	1822
(22 AND BUTANOL).USPT,JPAB,EPAB,DWPI.	6
(L22 AND BUTANOL).USPT,JPAB,EPAB,DWPI.	6

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L23: Entry 6 of 6 File: USPT Jun 23, 1992

DOCUMENT-IDENTIFIER: US 5124246 A
TITLE: Nucleic acid multimers and amplified nucleic acid hybridization assays using same

Detailed Description Text (25):
The multimer is then added to the solid phase-analyte-probe complex under hybridization conditions to permit the multimer to hybridize to the available second binding sequences of the amplifier probe of the complex. The resulting solid phase complex is then separated from any unbound multimer by washing. The labeled oligonucleotide is then added under conditions which permit it to hybridize to the complementary oligonucleotide units of the multimer. The resulting solid phase labeled nucleic acid complex is then separated from excess labeled oligonucleotide, by washing to remove unbound labeled oligonucleotide, and read.

Detailed Description Text (62):
Again the tube was vortexed and then incubated at room temperature overnight. The reaction mixture was extracted with n-butanol to a volume of 100 .mu.l, 100 .mu.l of 3.times.stop mix (25% glycerol, 0.05% bromphenol blue, 0.5% sodium dodecyl sulfate, 25 mM EDTA) was added and then heated to 100.degree. C. for 5 min. to denature the sample. Twenty .mu.l portions of this solution were then added to the wells of a 7% denaturing polyacrylamide gel (10 cm.times.10 cm.times.1.5 mm). The gel was run at 10 V/cm until the bromphenol blue dye was within 0.5 cm of the bottom of the gel. The products formed were made visible by placing the gel on a thin layer chromatography plate containing a UV fluorescing dye covered with Saran wrap and illuminating with a long wave UV lamp. The products absorb the UV irradiation and cast a visible shadow. Bands of increasing length were observed and polymeric products greater than twenty oligomeric units long were cut out. These products were eluted from the gel by soaking in Maxim-Gilbert buffer (0.1 M Tris-HCl, pH 8, 0.5 M NaCl, 5 mM EDTA).

Detailed Description Text (72):
A sample of 0.2 OD260 units of either fragment was dissolved in 0.5 .mu.l of 0.1 M sodium borate, pH 9.3, to which 9.5 .mu.l of DITC in dimethylformamide (2 mg/ml) was added. The solution was vortexed and set at room temperature overnight in the dark. After the addition of 300 .mu.l of n-butanol and mixing, 300 .mu.l of water was added. The mixture was vortexed and centrifuged to separate the layers. The sample was extracted several times until the aqueous phase was lowered to a volume of approximately 50 .mu.l and then vacuumed to dryness. The polymer was then treated with 10 .mu.l of 1 M glycine, pH 9.5, for 2 hr to modify any remaining isothiocyanate groups. The mixture was loaded onto a 10 ml Sephadex G-25 column, eluted with water, collected, evaporated to dryness, taken up in 1% SDS and loaded onto a 7% polyacrylamide gel (vertical 2% agarose gels are preferred for preparation runs). The gel was run at 60 ma and then stained with ethidium bromide. Bands estimated to comprise 10-25 units of the oligomer were cut, electroeluted and precipitated.

Detailed Description Text (112):
An 18 base oligomer, 5'-XGGTCCTAGCCTGACAGC-3', where X is defined as above, was synthesized, modified with DITC in 95:5 (v/v) dimethylformamide:0.1 M sodium borate, pH 9.3, extracted with n-butanol, and combined with horseradish peroxidase (HRP).

Detailed Description Text (125):
Label oligonucleotides were derivatized with alkaline phosphatase (AP) as follows. Calf intestinal AP (3 mg in buffer; immunoassay grade, Boehringer-Mannheim) was placed in a Centricon 30 Microconcentrator. Approximately 2 ml of 0.1 M sodium borate, pH 9.5, was then added and the device was spun at 3500 rpm until a final volume of 40

.mu.l was obtained. The alkylamino oligonucleotide (Section 3C) was then activated with DITC, extracted with butanol, and combined with the protein as described for the HRP probe. PAGE, elution (with 0.1 M Tris, pH 7.5, 0.1 M NaCl, 10 mM MgCl.sub.2, 0.1 mM ZnCl.sub.2), and concentration as described for the HRP conjugates were employed. The final product was stored at 4.degree. C.

☐ Generate Collection

L16: Entry 9 of 23 File: USPT Apr 14, 1998

DOCUMENT-IDENTIFIER: US 5739022 A
TITLE: Copper(II) nuclease compounds, compositions, and kits

Brief Summary Text (4):
There has recently been considerable interest in the development of chemical nucleases, i.e., synthetic nonenzymatic reagents that can recognize and cleave specific nucleic acid structures or sequences. Because of the intimate role of metal ions in enzymatic cleavage reactions, research has turned to developing metal ions or metal ion complexes that can cleave nucleic acids.

Brief Summary Text (12):
Hydrolytic cleavage of DNA has also been reported. Basile et al., J. Amer. Chem. Soc., 109 (1987) 7550-7551, reported DNA hydrolytic cleavage with a metal complex, (Ru(DIP).sub.2 Macro.sup.n+) (DIP=4,7-diphenyl-1,10-phenanthroline; Macro=4-7-[(NH.sub.2 CH.sub.2 CH.sub.2).sub.2 NCH.sub.2 CH.sub.2 NSO.sub.2 C.sub.6 H.sub.4]-1,10-phenanthroline) where the DIP ligands serve to direct the complex to the DNA and the Macro ligand contains chelate arms to bind divalent metals and promote hydrolysis. This hydrolytic cleavage was achieved at pH=8.5 in the presence of Co.sup.2+, Cu.sup.2+,Zn.sup.2+, Cd.sup.2+ and Pb.sup.2+. Morrow et al., Inorg. Chim. Acta, 195 (1992) 245-248, reported cleavage of DNA by nickel complexes; however, there appeared to be evidence that the cleavage is oxidative. DeRosch et al., Inorg. Chem., 29 (1990) 2409-2416, reported that M(bpy).sup.2+ and M(tren).sup.2+, (M=Ni(II) or Cu(II)) were generally not effective for nicking supercoiled plasmid DNA; however, Cu(bpy).sup.2+ was observed to cleave the plasmid DNA oxidatively.

Detailed Description Text (14):
Surprisingly, the unusual geometry of the complexes of formula (I) favors hydrolytic cleavage of nucleic acids. The five coordinate geometry about the copper(II) is not seen in other metal complexes which tend to have octahedral or tetrahedral symmetries. Copper(II), in contrast, tends to have a square pyramidal geometry. The small chelate ring of L.sup.1, particularly triazacyclononane, favors the Cu(II) oxidation state, because the ligand cannot achieve the tetrahedral coordination geometry preferred by Cu(I). This effect has been observed in Cu([9]aneN.sub.3)Cl.sub.2, which is irreversibly reduced in aqueous solution at 100 mV versus SCE. The low potential and the irreversibility of the reduction process indicate that the ligand destabilizes the Cu(I) oxidation state. Many metal-based DNA cleavage agents rely on activation of, for example, dioxygen or hydrogen peroxide by a reduced metal ion, and this mechanism is not possible for Cu([9]aneN.sub.3)Cl.sub.2.

Detailed Description Text (22):
Cleavage of RNA and single-stranded DNA by Cu([9]aneN.sub.3)Cl.sub.2 has been demonstrated. The extent of cleavage of nucleic acids increases with increasing concentration of metal complex and with increasing reaction time. The concentration of Cu(II) complex is preferably in the range of 0.1 mM to 10 mM. Cleavage does not occur significantly in the absence of metal complex, although DNA requires a higher ratio of metal complex to substrate to achieve cleavage in a reasonable time period. The pH of the reaction medium is preferably near-physiological pH, i.e., pH =7.0 to 8.5, and maintained by a noncoordinating buffer. For RNA, the reaction is preferably carried out at about pH 7.2, because at pH above 7.5, the RNA is spontaneously degraded. The temperature of the reaction ranges from about 25.degree. C. to about 55.degree. C. The preferred reaction temperature is 37.degree. C. for RNA and 50.degree. C. for DNA. At higher temperatures, RNA spontaneously degrades. Significant cleavage is seen after about 6 hours for RNA and about 12 hours for single-stranded DNA.

Detailed Description Text (26):
Time- and concentration-dependent cleavage of single-stranded plasmid M13mp18 DNA has

been demonstrated, i.e., the DNA cleavage is due to the presence of the metal complex in accordance with the present invention. The cleavage was insensitive to the presence of catalase, superoxide dimutase, methanol (as a radical trap), or to the exclusion of air, suggesting that cleavage was occurring via a nonoxidative pathway. A DNA hairpin, analogous to the RNA hairpin described herein, showed no cleavage in the double-stranded region even after one week at 50.degree. C., although the single-stranded region was cleaved. This result indicates that single-stranded DNA is a substrate for cleavage by Cu([9]aneN.sub.3)Cl.sub.2.

Detailed Description Text (68):
The oligonucleotide 5'-UCCCCCUCUUCGGAGGGGGA-3' (SEQ.ID NO.1) was 5'-end labelled with radioactive .sup.32 P and allowed to anneal to itself to form a hairpin loop (loop region underlined). The oligonucleotide was synthesized on an Applied Biosystems 392 DNA/RNA Synthesizer available from Applied Biosystems of Foster City, Calif., U.S.A., using the procedure outlined in User Bulletin #69 pp. 1-15 (October 1992). The labeling of the 5' end and annealing was carried out according to the general methods described in Molecular Cloning, A Laboratory Manual, 2nd ed., J. Sambrook, E. F. Fritsch, and T. Maniatis, Cold Spring Harbor, Cold Spring Harbor Press (1989).

Detailed Description Text (106):
Unincubated M13mp18 DNA was observed as a single band on the agarose gel while M13mp18 that was degraded was observed as a smear under the band: the greater the extent of cleavage, the smaller the fragments and the farther the smear is under the band. Although some degradation of the DNA occurred in the absence of the metal complex (e.g., comparing lanes 6, 11 and 16 with lane 1) at 12, 24 and 48 hours, substantial enhancement of the rate of cleavage of the DNA occurred in the presence of the metal complex, particularly at the higher concentrations. This enhancement was both time and copper complex concentration dependent. Cleavage was seen as early as 6 hours; however, the greatest extent of cleavage is seen in the DNA treated for 48 hours with 1.0 mM Cu([9]aneN.sub.3)Cl.sub.2 (lane 20).

Detailed Description Text (109):
Modified phenanthridines containing appended metal chelates are synthesized to cleave double-stranded DNA. To accomplish the functionalization of methidium or ethidium, a functionalized triazacyclononane ligand is synthesized according to the following procedures. 1,4-ditosyl-1,4,7-triazacyclononane is first prepared as described by A. E. Martin et al. (J. Org. Chem., 47 (1982) 412). 1-allyl-1,4,7-triazacyclononane is then prepared from the 1,4-ditosyl-1,4,7-triazacyclononane and allyl chloride by reaction in DMF (dimethylformamide) and the double bond is converted to the alcohol by hydroboration and hydrolysis according to standard protocols (see, Larock, Richard G., Comprehensive Organic Transformations: A Guide to Functional Group Preparations, VCH Publishers, Inc., New York, N.Y. (1989)). Oxidation of the alcohol (see, Corey, S., Tet. Lett., (1975) 2647) provides the aldehyde which is used to modify the phenanthridine on the phenyl ring by the method of Hertzberg and Dervan (R. P. Hertzberg and P. B. Dervan, Biochem., 23 (1984) 3934-3945) to prepare the bifunctional intercalator-chelate shown in FIG. 5A. Alternatively, the method of Keck and Lippard (Tet. Lett., 34 (1993) 1415-1416) is used to attach triazacyclononane to the exocyclic amine of methidium to make the bifunctional intercalator-chelate shown in FIG. 5B. Because Cu([9]aneN.sub.3)Cl.sub.2 hydrolyzes amides, the amide linkages are reduced by standard methods (Larock, Comprehensive Organic Transformations, supra, p. 432). The copper complexes, shown in FIGS. 5A' and 5B', of the respective intercalator-chelates are prepared by reaction of the ligand with an appropriate copper salt, such as CuBr.sub.2, in water and precipitated with ethanol, as described previously. The copper complexes are characterized by standard methods of IR, NMR, ESR spectroscopy and by standard techniques of elemental analysis.

Detailed Description Text (112):
The ability of phenanthridine-Cu([9]aneN.sub.3)Cl.sub.2 to nick supercoiled plasmid DNA is compared with its ability to cleave poly(dA.dT), available from Sigma Chemical Co., St. Louis, Mo., U.S.A. End labelled 5' .sup.32 P-poly(dA.dT), is prepared by using the technique to radiolabel the 5' end with .sup.32 P as previously described in Example 10. The end labelled 5' .sup.32 P poly(dA.dT) is incubated with phenanthridine-Cu([9]aneN.sub.3)Cl.sub.2, Cu([9]aneN.sub.3)Cl.sub.2, and phenanthridine, and cleavage assayed on sequencing gels. Comparison of the band positions on the sequencing gels with those of Maxam-Gilbert sequencing lanes are made

to determine whether the cleavage products have only phosphate and hydroxyl termini. The reaction mixtures are also assayed by HPLC for evidence of the release of thymine and adenine. The absence of free bases and additional bands on the sequencing gels suggests that cleavage is hydrolytic.

Detailed Description Text (114):
The results show that cleavage of double-stranded DNA is achievable, when the metal complex is delivered appropriately to the double-stranded DNA molecule by phenanthridine-Cu([9]aneN.sub.3)Cl.sub.2.

Detailed Description Text (117):
The ability of Cu([9]aneN.sub.3)Cl.sub.2 and other compounds in accordance with the present invention for hydrolytic decomposition of phosphate-derivative anticholinesterases such as insecticides is determined. The relatively nontoxic analogs, O-ethyl S-ethyl methylphosphonothiolate, and diethyl-thiophosphate, are used in these experiments as models for the hydrolytic decomposition of the phosphate-derivative anticholinesterases such as thiophosphate ester insecticides, e.g., parathion and malathion. The phosphonothiolate or thiophosphate ester (0.1 mM-10 mM) is reacted with Cu([9]aneN.sub.3)Cl.sub.2 (0.1 mM-1 mM) in ethanol and/or water solvent at pH 7.0-10.0, maintained with HEPES buffer, and an ionic strength of 0.1M, maintained with NaNO.sub.3, at 50.degree. C. for 90 minutes to three hours. It is noted that the concentration of the organo-phosphate is greater than the concentration of the Cu(II) complex for effective catalysis. As the pH increases, the rate of competing noncatalytic reactions increases. It is also noted that an extremely high ionic strength will slow the reaction. The temperature of the reaction is 50.degree. C. or higher. It is useful to note that higher temperatures will increase the reaction rate without decomposing the Cu(II) complex. The minimum reaction time is about 90 minutes.

Detailed Description Text (118):
The reaction is monitored by .sup.31 P and .sup.1 H NMR spectroscopy, and the products identified by their characteristic NMR spectra. The rate of reaction in the presence and absence of the metal complex is determined by integration of the product NMR signals, and the rate enhancement is assessed. The order of reaction with respect to substrate and product is determined by varying the concentrations of the appropriate reagent over at least one order of magnitude. Turnover by the metal complex is demonstrated by reaction of an excess of the phosphodiester substrate with the metal complex and the production of greater than stoichiometric amounts of product. The results of these experiments show that Cu[9]aneN.sub.3 Cl.sub.2 is a catalyst for the hydrolysis of thiophosphate and phosphonothiolate esters and is applicable to the decomposition of insecticides with similar functionalities.